

Bioremediation and Biodegradation

The Effect of Five Forage Species on Transport and Transformation of Atrazine and Isoxaflutole (Balance) in Lysimeter Leachate

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ABSTRACT

A field lysimeter study with bare ground and five different ground covers was established to evaluate the effect of forage grasses on the fate and transport of two herbicides in leachate. The herbicides were atrazine (ATR; 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and isoxaflutole [IXF; 5-cyclopropyl-4-(2-methylsulfonyl-4-(trifluoromethyl-benzoyl)isoxazole)], which has the commercial name Balance (Aventis Crop Science, Strasbourg, France). The ground covers included orchardgrass (*Dactylis glomerata* L.), smooth brome grass (*Bromus inermis* Leyss.), tall fescue (*Festuca arundinacea* Schreb.), timothy (*Phleum pratense* L.), and switchgrass (*Panicum virgatum* L.). The results suggested that the total IXF (parent + metabolites) showed higher mobility than ATR and its metabolites. Differences in the timing of transport reflected the rapid degradation of IXF to the more soluble, stable, and biologically active diketetonitrile (DKN) metabolite in the system. Although grass treatments did not promote the hydrolysis of DKN, they significantly reduced its transport in the leachate through enhanced evapotranspiration. Grass treatments significantly enhanced ATR degradation in the leachates and soils, especially through N dealkylation, but they did not reduce total ATR transported in the leachate. Leachate from the orchardgrass lysimeters contained the highest proportion of ATR metabolites (64.2%). Timothy and smooth brome grass treatments also displayed a significant increase in ATR metabolites in leachate. Grass-treated lysimeters showed higher microbial biomass carbon than bare ground. For ATR treatments, the proportion of metabolites in the leachate strongly correlated with the elevated soil microbial biomass carbon in forage treatments. In contrast, DKN degradation was poorly correlated with soil microbial biomass carbon, suggesting that DKN degradation is an abiotic process.

HERBICIDES are among the most important nonpoint-source pollutants in the Midwestern United States. More than 70% of the herbicides used in the USA are applied in the Midwest for corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production (USDA, 2001). The USDA reports that about 24.6 million kg of ATR are applied to the soil annually, more than any other herbicide (USDA, 2001). In 2000, it was applied on more than 68% of all corn acreage. Atrazine and its metabolites were found in 24% of 579 wells monitored

by the USDA in 1993 (USDA, 1993). In another ground water study, pesticides and their metabolites were analyzed in 303 different wells from 12 Midwestern states (Kolpin et al., 1996). Atrazine and its degradation products, deethylatrazine (DEA) and deisopropylatrazine (DIA), represented three of the nine most frequently detected compounds, and only the alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] metabolite, alachlor-ethanesulfonic acid (ESA), was detected more frequently than ATR and DEA.

In a study encompassing 141 streams throughout the Midwestern USA, Lerch et al. (1998) reported that ATR was detected in all 46 streams tested in 8 midwestern states, and in all 95 streams tested in northern Missouri during the corn post-plant period. The ATR metabolites, hydroxyatrazine (HA), DEA, and DIA, were detected in 87 to 100% of the samples collected at post-plant (Lerch et al., 1998). In addition, other monitoring studies of Midwestern streams have shown that ATR and its metabolites are detected at their highest levels during the spring flush period (Thurman et al., 1992; Lerch et al., 1995), and ATR, DEA, and HA are generally detected year-round. Of the commonly used agricultural pesticides, ATR and its metabolites are, overall, detected more frequently and at higher concentrations in surface waters of the Midwestern USA than any other pesticide transported from a nonpoint source. In surface water, ATR contamination of streams has ranged from 80 to 300 µg/L and could be detected for a 45- to 60-d period following application (USDA, 1994). Extremely high concentrations of 250 to 1500 µg/L ATR have been measured in the first runoff and in topsoil along tested waterways adjacent to corn fields (USDA, 1994, 1995). This is far above the USEPA maximum contaminant level of 3 µg/L for drinking water.

The herbicide Balance (isoxaflutole; IXF) belongs to a new family of herbicides referred to as isoxazoles. It was originally developed by Rhône-Poulenc Agro and now marketed by Aventis Crop Science. This family of herbicides inhibit HPPD (4-hydroxy-phenylpyruvate dioxygenase) in both C3 and C4 plants. This inhibition blocks carotenoid biosynthesis and leads to an absence of chloroplast development (Pallett et al., 1998). In field trials, IXF has been reported to effectively control a wide spectrum of triazine-resistant weeds in corn production. It has the advantage of low application rates (11–64 g/ha) and season-long control (Lazo et al., 1997;

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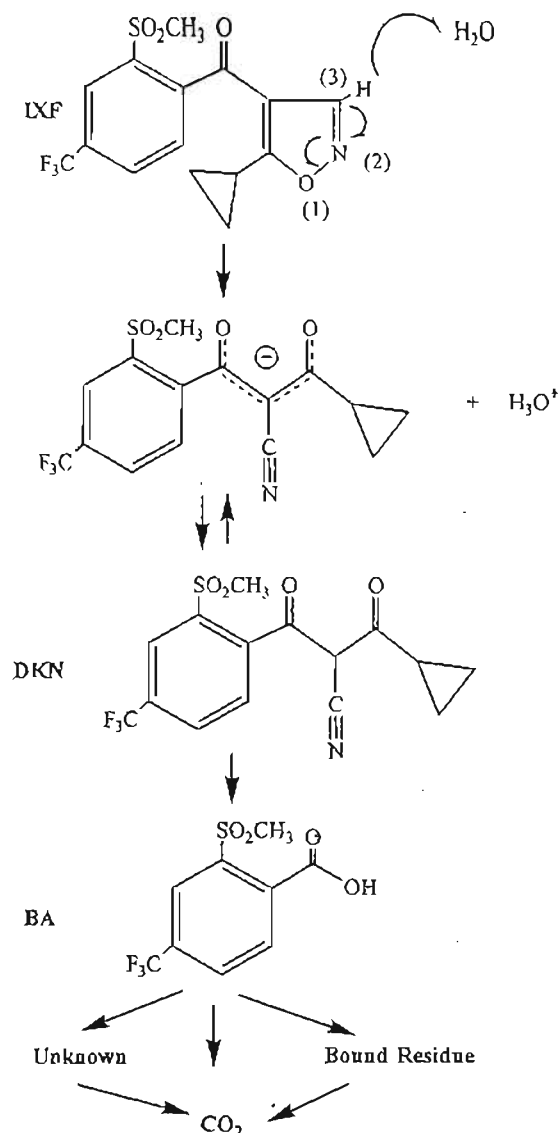
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Abbreviations: ATR, atrazine; BA, benzoic acid; DEA, deethylatrazine; DIA, deisopropylatrazine; DKN, diketetonitrile; HA, hydroxyatrazine; IXF, isoxaflutole.

Menendez et al., 1997, 1998). It received conditional regulatory approval from the USEPA in 1998 and was commercially introduced for the 1999 growing season in 16 U.S. corn-producing states. Isoxaflutole does not have inhibitory effects on HPPD and it has a very short soil half-life of less than three days (Pallett, 2000). To possess herbicidal activity, IXF must be transformed to a diketonitrile (DKN) metabolite in soils (Pallett et al., 1998). Diketonitrile is more stable than the parent compound and is moderately water-soluble. Further degradation of the DKN metabolite produces a nonbiologically active benzoic acid (BA) metabolite, a highly stable and water-soluble compound (Fig. 1). Isoxaflutole is sensitive to hydrolysis by light, heat, and high pH conditions (Lin, 2002; Beltran et al., 2000). This herbicide is reported to be less toxic to mammals than ATR (USEPA, 1998a,b). It is classified under Toxicity Category IV, which is defined as an acute oral toxicity (LD_{50}) in rats of >5000 mg/kg. Isoxaflutole has potential for wide-spread use and may significantly lower ATR usage in the next decade. Within the first three years of introduction, it was applied to an estimated 4 to 23% of corn acreage in Midwestern states (USDA, 2001). Therefore, DKN and BA would be expected to appear as nonpoint-source pollutants in the near future.

Among several management practices, a well-designed tree-shrub-grass riparian buffer strip is recognized as one of the most cost-effective approaches to mitigate nonpoint sources of agricultural pollutants derived from cropland (Schultz et al., 1991, 1995; Lowrance et al., 1997). In a watershed study conducted in central Texas, a 44 to 50% reduction in ATR levels was observed when a filter strip was used (Hoffman et al., 1995). There are several physical, chemical and biological mechanisms involved in the process of bioremediation within the riparian buffer zone. Organic pesticides can be intercepted by the roots and residue of the vegetation via physical adsorption and filtration (Hoffman et al., 1995; Pestemer et al., 1984). Bacteria growing in the root zone may have the capacity to metabolize herbicides through various biochemical mechanisms including enzymatic oxidation and hydrolysis (Ambus, 1993; Mandelbaum et al., 1993, 1995; Struthers et al., 1998). Direct plant uptake may also help to eliminate the agrochemicals from subsurface flow (Burken and Schnoor, 1997). Furthermore, the improvement of soil characteristics by vegetation (e.g., increases in organic matter content and improved porosity) may enhance the rhizosphere's capacity for adsorption and abiotic transformation of pollutants (Mandelbaum et al., 1993; Martin-Neto et al., 1994).

To establish a successful tree-shrub-grass vegetative riparian buffer zone for bioremediation, the selected ground cover species must be able to grow satisfactorily in the shade cast by the trees along the streambank. In addition, the ground cover must possess the capacity to adsorb and hold or further degrade the agricultural chemicals. Information derived from a shade screening trial has helped to identify ground cover components for buffer zones (Lin et al., 1998). The major objective of the work reported below was to quantify the effect of five forage species on transport and transformation of



	Half-life in soil (days)	Partition coefficient K_{oc}	Solubility in water (mg/L)
IXF	<1.4	122	6.2
DKN	8-16	92	300
BA	20-60	69	8000

Fig. 1. Degradation pathway and chemical properties of isoxaflutole (IXF) and its metabolites (Beltran et al., 2000; Rhône-Poulenc, personal communication, 2000). The term K_{oc} is the organic carbon partition coefficient.

ATR and IXF in lysimeter leachate. An additional objective was to compare the temporal losses of these herbicides to leachate after major rainfall events.

MATERIALS AND METHODS

Experimental Design

Thirty-six lysimeters (0.5 m deep \times 1 m in diameter) with bare ground and five different ground covers were established for ATR and IXF treatments in 1998 at the University of Missouri Horticulture and Agroforestry Research Center,

New Franklin, MO (92°46' W, 39°1' N). The ground covers included orchardgrass, smooth bromegrass, tall fescue, timothy, and switchgrass. Orchardgrass, tall fescue, timothy, and smooth bromegrass are cool-season forages that possess the C3 photosynthetic pathway. Switchgrass is a warm-season C4 plant. Lysimeters were arranged as a randomized block design with three replications of each ground cover per herbicide treatment.

Each lysimeter was filled with a sandy loam soil with an average pH of 7.0, organic matter content of 0.72%, and cation exchange capacity of 3.0 cmol/kg. The lysimeter soil had no history of ATR or IXF application (<20 ng/L). The interior surface of the lysimeters was fluorinated and each lysimeter was attached to a 5-cm rigid PVC drain line that ended at a central collection facility. Lysimeters were manually seeded in August 1997 and reseeded in February 1998 to achieve uniform vegetation coverage. In September 1998, when all species were vigorous and well established, herbicide solutions were uniformly applied to each lysimeter with a graduated cylinder containing 3 L of ATR (500 µg/L) or IXF (80 µg/L). Care was taken to avoid bringing foliage into contact with the herbicide solutions. The applied concentrations are representative of those expected in the surface runoff under corn-growing conditions in northern Missouri (USDA, 1995). Leachate from each lysimeter was collected in a 13-L Nalgene high density polypropylene carboy (Catalog no. 02-960-20C; Fisher Scientific, Pittsburgh, PA). Samples were collected after each major rainfall event for a period of 25 d (Fig. 2).

Analysis of Atrazine and Selected Metabolites

The concentrations of ATR and its metabolites DEA, DIA, and HA in the leachate were determined by solid-phase extraction (SPE) followed by reversed phase high performance liquid chromatography (HPLC) with UV detection as previously reported (Lerch and Donald, 1994; Lerch et al., 1995). A 100-mL aliquot was subsampled from the leachate and

filtered through a 0.45-µm nylon filter. Then, the samples were spiked with 100 ng of the surrogate compound terbutylazine (TRB). Spiked samples were passed through a preconditioned LRC 500-mg silica-based C₁₈ resin solid-phase extraction cartridge (Varian, Harbor City, CA) and were eluted with 5 mL of 9:1 methanol (CH₃OH) and 0.5 M potassium phosphate (KH₂PO₄) buffer, pH 7 to 7.5. The phosphate buffer prevents mixed-mode binding of HA to the C₁₈ resin (Lerch et al., 1997). The eluate was concentrated to dryness under a stream of N₂ at 30°C and reconstituted with 1 mL of 40% CH₃OH. Reconstituted samples were analyzed by C₈ reverse-phase HPLC-UV as described by Lerch et al. (1995). The HPLC conditions were as follows: column temperature was maintained at 35°C; injection volume was 40 µL; and UV detection of all analytes was at 220 nm. The mobile phase consisted of acetonitrile (ACN) and distilled deionized water at a flow rate of 1.45 mL/min. The mobile phase gradient was used as follows: 15% ACN for 15 min; 30% ACN for 13 min; 40% ACN for 9 min; 85% ACN for 9 min; and reequilibration at 15% ACN for 20 min. Retention times were 5.9, 11.7, 13.3, 26.2, and 35.4 min for DIA, DEA, HA, ATR, and TRB, respectively. The limit of quantitation (LOQ) was 0.02 µg/L for ATR, DEA, and DIA and 0.05 µg/L for HA.

Analysis of Isoxaflutole and Its Metabolites

The analysis of IXF and its metabolites, DKN and BA, was performed by solid-phase extraction followed by reversed phase HPLC-mass spectrometry (HPLC-MS). Complete details of the analytical procedures and instrument conditions are described by Lin et al. (2002). In brief, leachate samples of 100 mL were acidified with formic acid (HCOOH) (0.6%, pH 2.1) to stabilize IXF, and 1 ng of the surrogate, 2,4-D (2,4-dichlorophenoxyacetic acid), was spiked into each sample for recovery correction. Sample solutions were passed through preconditioned Isolute Env+ 200 mg polystyrene divinylbenzene polymer solid-phase extraction cartridges (International

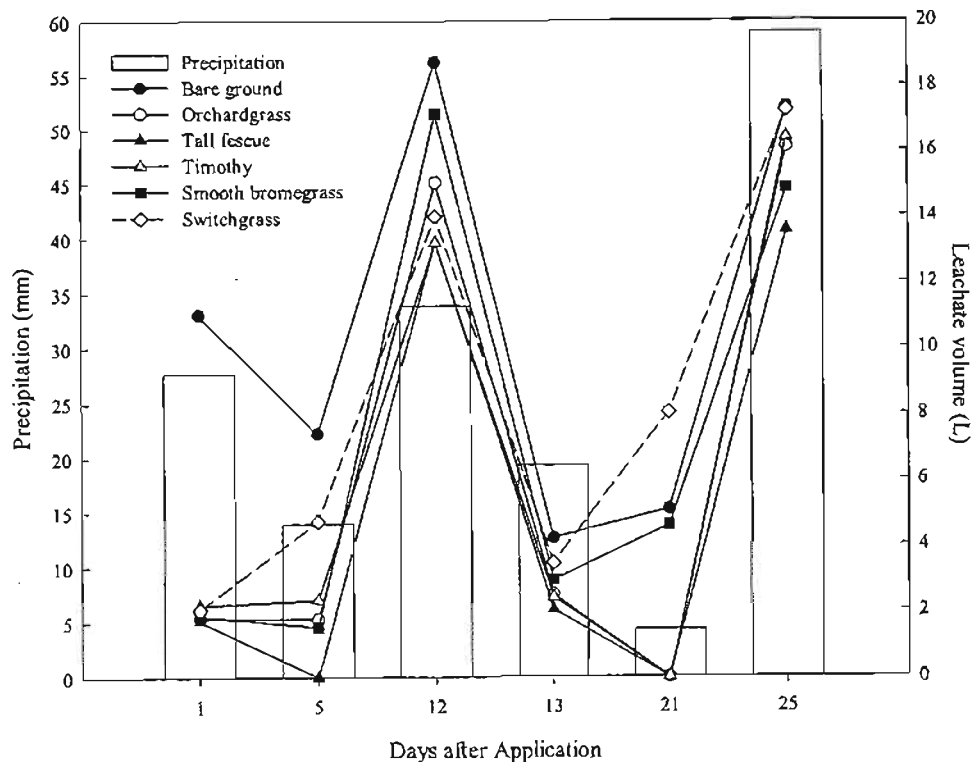


Fig. 2. Precipitation (mm) and leachate volume (L) collected from each ground cover treatment.

Sorbent Technology-Jones Chromatography Inc., Lakewood, CO) at a flow rate of 4 mL/min. Cartridges were then rinsed with 3 mL of distilled deionized water and eluted with 30 mL of 100% ACN at a flow rate of 2 mL/min. Eluates were rotovaporated and further concentrated to approximately 50 μ L under a stream of N_2 at 40°C. The final extract was reconstituted to 150 μ L with 1:9 ACN and 1.3% HCOOH solution (pH 2.1). The analytes were quantified by C_{18} reverse-phase HPLC-MS using a Hewlett-Packard (Palo Alto, CA) 1100 HPLC unit coupled to a Hewlett-Packard 1100 mass selective detector. The ion source was operated in the negative ion mode. Characteristic ions used for analysis were m/z 159 for BA and m/z 358 for IXF and DKN. The mobile phase consisted of CH_3OH and distilled deionized water at a flow rate of 0.8 mL/min. A mobile phase gradient was used as follows: 40% CH_3OH for 1 min followed by a ramp to 80% CH_3OH from 8 min to 28 min. Retention times were 8.9, 9.4, 12.2, and 12.8 min for BA, DKN, IXF, and 2,4-D, respectively. The LOQ for IXF, DKN, and BA was 50 ng/L.

Determination of Microbial Biomass Carbon

Soil microbial populations in each lysimeter were evaluated by measuring soil microbial biomass carbon. Biomass carbon was determined by a modified chloroform ($CHCl_3$) fumigation and direct extraction method (Jordan and Beare, 1991). Twenty-gram soil samples from each lysimeter were adjusted to 0.10 g H_2O per g dry soil. These samples were derived from a mixture of five soil cores of approximately 2×35 cm each. Samples were either fumigated with $CHCl_3$ at room temperature (approximately 25°C) under vacuum for 24 h or not fumigated. Fumigation allows for the subsequent extraction of microbial carbon. Unfumigated samples were stored at 4°C in a standard refrigerator in a closed specimen cup. Biomass carbon from fumigated and unfumigated samples was extracted by adding 80 mL of 0.5 M potassium sulfate (K_2SO_4) and shaking for 30 min on a rotary shaker at 320 rpm. The liquid fraction was filtered through Whatman (Maidstone, UK) #1 filter paper into a specimen cup. Biomass carbon was evolved as CO_2 by potassium persulfate ($K_2S_2O_8$) digestion (Jordan and Beare, 1991). Carbon dioxide was trapped into 1 mL of 0.1 M NaOH. A volume of 100 mL of 0.5 M barium chloride ($BaCl_2$) was added following CO_2 trapping. This solution was then titrated with 0.01 M HCl containing 1% phenol-

phthalein as an indicator (pink) until a colorless endpoint was reached. The amount of trapped carbon was determined from a standard curve constructed by $K_2S_2O_8$ digestion of glucose standards (0, 100, 200, 300, 400, and 500 mg carbon). Soil microbial biomass carbon was calculated as the difference between duplicate fumigated and unfumigated samples using a mineralization conversion factor of 0.41 (Voroney and Paul, 1984) for the lysimeter soil.

RESULTS AND DISCUSSION

Leaching of Isoxaflutole and Atrazine through Lysimeters

Six rainfall events, with total precipitation ranging from 5 to 58 mm, occurred within a 25-d period after herbicide application (Fig. 2). Leachate volume was generally proportional to the amount of precipitation, with greatest volume observed on the third (12 d after application) and sixth (25 d after application) rainfall events. As expected, the grass-treated lysimeters generally showed less leachate volume than the control treatment due to their higher evapotranspiration (Fig. 2). Total ATR losses (i.e., ATR + DEA + DIA + HA) to lysimeter leachate were greatest for the two largest rainfall events (Fig. 3). This is also evident in individual plots of ATR, DEA, DIA, and HA losses versus time (Fig. 4). In contrast, total losses of IXF (i.e., IXF + DKN + BA) to leachate were greatest for the first three events (Fig. 3 and 5). Differences in the timing of transport reflect the rapid degradation of IXF to the more soluble DKN and BA metabolites (Fig. 5) as compared with ATR. A range of 0.06 to 0.49% of the applied ATR was collected in the leachate during the first rainfall event (Fig. 3). In contrast, 1.40 to 8.36% of the applied IXF was collected in leachate during the first rainfall event (Fig. 3). As described above, this difference can be attributed to the rapid conversion of IXF to the highly soluble DKN and the greater sorption of ATR and its metabolites to soil colloids. In addition, only a

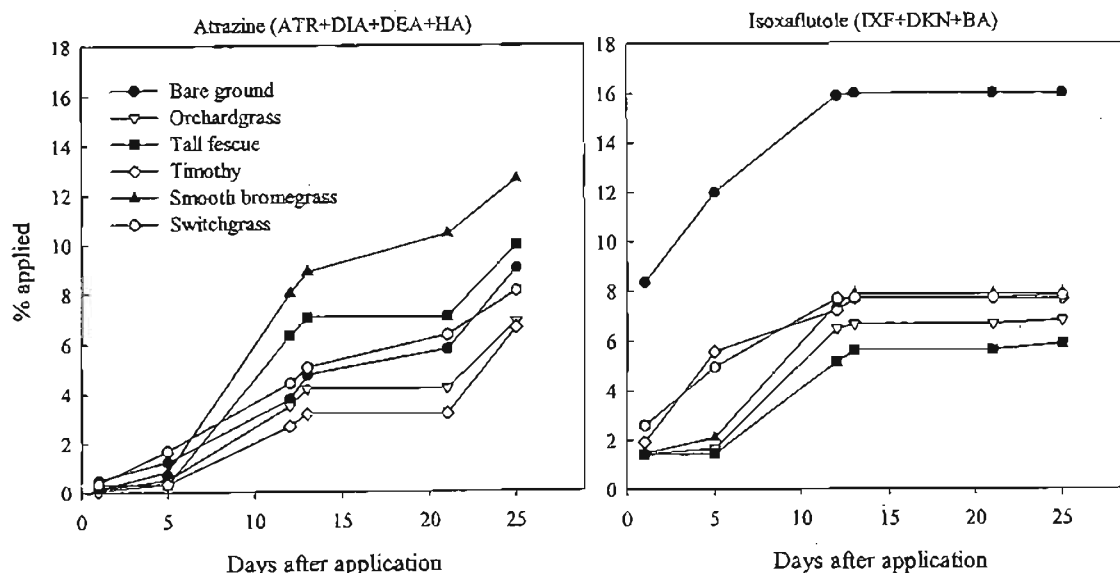


Fig. 3. Cumulative total atrazine and isoxaflutole (IXF) lost to leachate.

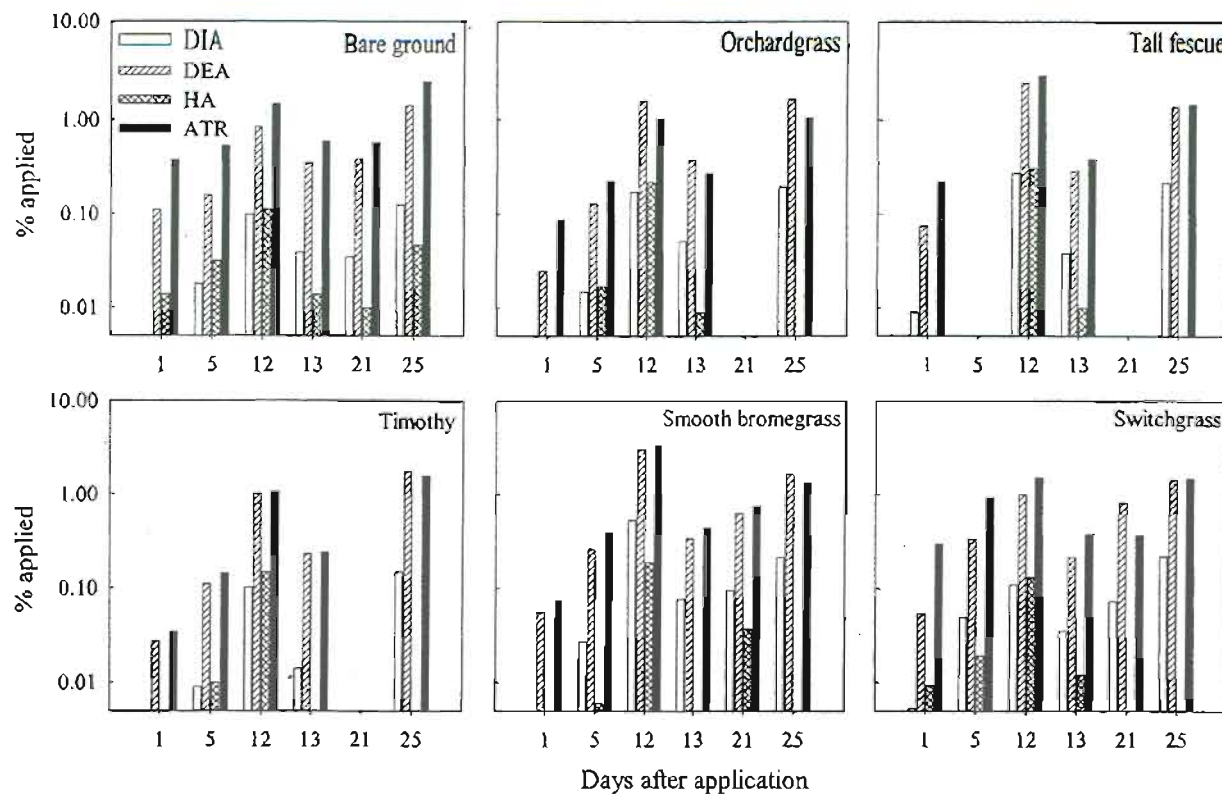


Fig. 4. Appearance of atrazine (ATR) and its metabolites deisopropylatrazine (DIA), deethylatrazine (DEA), and hydroxyatrazine (HA) in leachate over time.

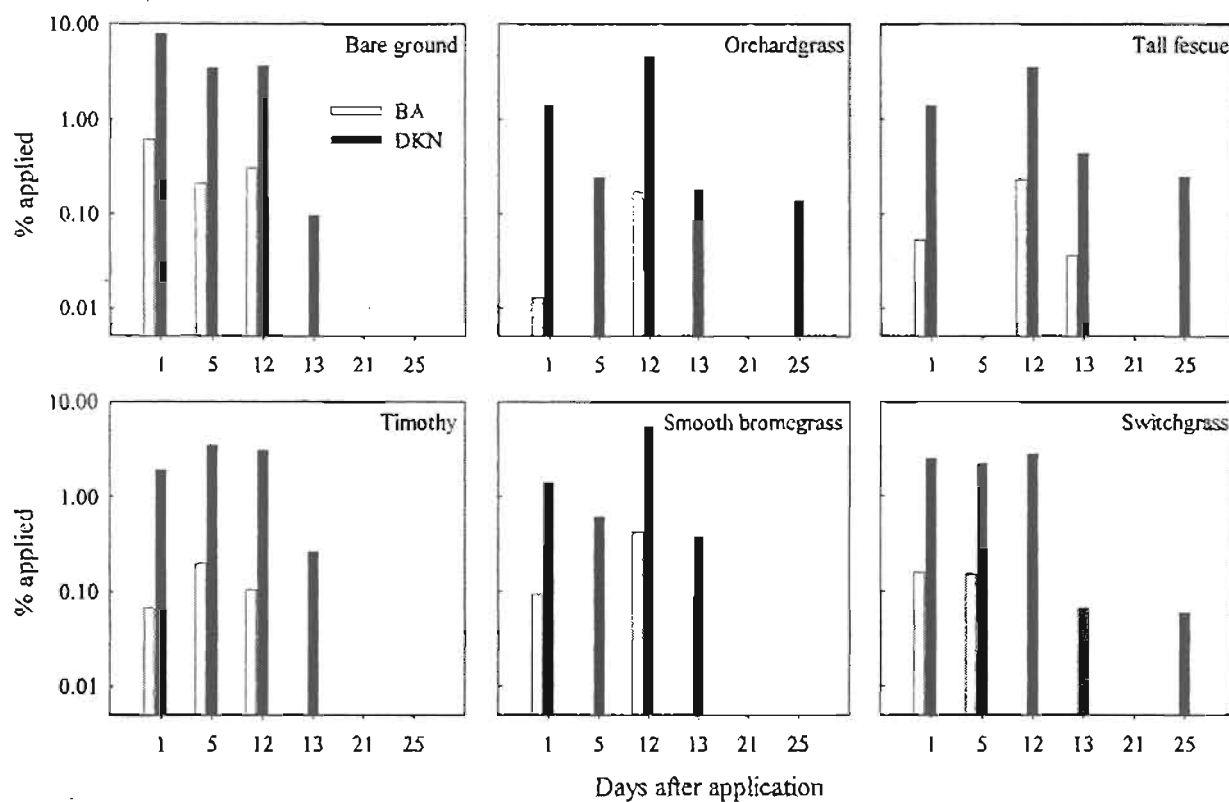


Fig. 5. Appearance of isoxaflutole (IXF) and its metabolites diketonitrile (DKN) and benzoic acid (BA) in leachate over time.

Table 1. Percentage losses of atrazine (ATR) and its metabolites deisopropylatrazine (DIA), deethylatrazine (DEA), and hydroxyatrazine (HA) to leachate after 25 d.

Ground treatment	ATR	DIA	DEA	HA	Total loss	Proportion of metabolites in leachate
	% of applied ATR					% of total loss
Bare ground	5.55a†	0.28b	2.95b	0.21a	8.99a	37.50c
Orchardgrass	2.52c	0.42b	3.68b	0.23a	6.85a	64.20a
Tall fescue	4.92ab	0.52b	4.16b	0.32a	9.92a	51.10bc
Timothy	3.04ab	0.27b	3.14b	0.16a	6.61a	58.71b
Smooth brome grass	5.87a	0.89a	5.61a	0.22a	12.58a	53.34b
Switchgrass	4.31ab	0.43b	3.19b	0.16a	8.08a	50.30bc
Mean	4.37	0.47	3.78	0.22	8.84	52.50

† Means followed by the same letter within the column do not differ significantly from each other at a significance level of 0.1 using Fisher's LSD test.

small portion of ATR was degraded into its more soluble metabolites one day after application (Fig. 3 and 4).

Diketonitrile and BA have pK_a values near 2.0 (Zeneca Agrochemicals [Richmond, CA], personal communication, 2000) making them highly vulnerable to leaching since they were in anionic form at the ambient soil pH (about 7) in this study. This, coupled with the high solubility of these metabolites, enhances the rate of their transport within the soil column, resulting in rapid loss to leachate during early rainfall events. Although major differences existed in the timing of transport through the lysimeters and the extent of degradation, relative total losses of ATR (8.84%) and IXF (8.59%) to leachate were not significantly different in this study (Tables 1 and 2). For each treatment, including bare ground, 100% of applied IXF found in the leachate was in the form of its metabolites, with DKN predominating (Fig. 5). The nonbiologically active BA accounted for only 0.18 to 1.12% of total IXF losses (Table 2). In contrast, of the total ATR lost to leachate, 37.50 to 64.20% was in the form of metabolites, with DEA the predominant metabolite (Table 1 and Fig. 4).

The fate of IXF reported in this study is consistent with previous laboratory and field observations (USEPA, 1998a,b). For instance, Pallett (2000) reported that the half-life of IXF degradation to DKN was from 12 to 24 h in soil under greenhouse conditions. Taylor-Lovell et al. (2000) also reported that the transformation of IXF to DKN is much faster in the soil than a soil-free system. They found that about 83% of IXF was transformed in the presence of soil after 24 h, but only 15% was transformed after 24 h in an aqueous solution. In a surface water monitoring program initiated by the Department of Natural Resources in Iowa (Iowa Department of Resources, Geological Survey Bureau, personal communication, 2000), DKN was reported to be the most frequently detected and predominant metabo-

lite in surface water near agricultural lands treated with IXF. The BA metabolite was the second-most dominant form of the herbicide found in the surface water. Parent IXF was detected in only 3 of 337 samples (0.8%) with average concentrations of only 0.012 $\mu\text{g/L}$. The USEPA has applied a mathematical model, the Screening Concentration in Ground Water Model (SCI-GROW), to predict the possible concentrations of IXF, DKN, and BA in ground water (USEPA, 1998b). Estimated maximum concentrations of IXF, DKN, and BA in ground water near corn fields are 0.00025, 0.23, and 6.1 $\mu\text{g/L}$, respectively.

After the final rainfall event, the percentage of applied ATR and metabolites in the leachate generally decreased in the order $\text{ATR} > \text{DEA} > \text{DIA} > \text{HA}$ in all treatments (Table 1). Several studies have shown that soil sorption intensity of ATR metabolites decreases in the order $\text{HA} > \text{DIA} > \text{DEA}$ in various types of soil (Brouwer et al., 1990; Moreau-Kervevan and Mouvet, 1998). For the metabolites, their mass present in the leachate coincided with their sorption intensity. However, in addition to the leaching potential, the mass of ATR and its metabolites available for leaching at a given time was also an important factor affecting their transport in the leachate. For instance, the greater mass of ATR present in the soils early in the study creates a large pool of ATR that is available for leaching, despite its stronger soil sorption compared with DEA and DIA (Moreau-Kervevan and Mouvet, 1998). This resulted in overall greater loss of the parent compound compared with its metabolites, especially for the first two rainfall events. Only the orchardgrass and timothy treatments showed greater loss of a specific metabolite (DEA) than parent ATR. The mass of ATR in the soil before each rainfall event, therefore, was the primary factor explaining its high concentration in the leachate after each event (Fig. 4, Table 1). Transport of DEA and DIA in

Table 2. Percentage losses of isoxaflutole (IXF) and its metabolites diketonitrile (DKN) and benzoic acid (BA) to leachate after 25 d.

Ground treatment	IXF	DKN	BA	Total loss	Proportion of BA in leachate
% of applied IXF					% of total loss
Bare ground	0.00	14.79a†	1.12a	15.91a	7.03a
Orchardgrass	0.00	6.46b	0.18b	6.64b	4.83a
Tall fescue	0.00	5.40b	0.31b	5.71b	7.37a
Timothy	0.00	7.37b	0.30b	7.67b	4.12a
Smooth bromegrass	0.00	7.35b	0.52b	7.87b	7.00a
Switchgrass	0.00	7.44b	0.31b	7.75b	3.98a
Mean	0.00	8.14	0.46	8.59	5.72

† Means followed by the same letter within the column do not differ significantly from each other at a significance level of 0.1 using Fisher's LSD test.

association with each rainfall event would also lower their mass for the following event and raise the proportion of ATR in the soil compared with the metabolites.

Hydrophobic bonding and H bonding are the major adsorption mechanisms for ATR, DEA, and DIA in most soil environments; however, these interactions are weaker for DEA and DIA (Cheng, 1990; Lerch et al., 1999; Moreau-Kervevan and Mouvet, 1998). At a soil pH of 7, ATR, DEA, and DIA are present only in neutral form due to their low pK_a values (less than 2). On the other hand, HA ($pK_a = 5.2$) binding to soils occurs by mixed-mode sorption, resulting from cation exchange and hydrophobic bonding (Lerch et al., 1997). Although the bulk soil pH was 7.0 in the lysimeters, the pH near soil colloids has been shown to be from 0.5 to 2 pH units below the bulk soil pH because of the concentration of protons near the negatively charged colloids (Weber, 1970). Therefore, 5 to 61% of the HA could be in a cationic form. The much stronger sorption of HA resulting from mixed-mode binding significantly limits the mass of HA transported in the lysimeter leachate compared with DEA and DIA (Fig. 4, Table 1).

In this experiment, both transformation and transport processes occurred simultaneously. Thus, it was not possible to clearly separate these two effects. The differential rates of transformation of these compounds also influenced their availability for leaching. For instance, the proportion of DEA and DIA relative to ATR depended on the rate of ATR transformation to DEA and DIA, rate of DEA and DIA degradation to other metabolites, and their differential rate of transport through the soil profile.

Effect of Forage Grasses on Herbicide Transport and Fate

Compared with the control, grass treatments reduced DKN transport by 51 to 64% (Table 2). The majority of the reduction was attributed to the lower leachate volumes of the grass treatments due to transpiration (Fig. 2). The control had significantly higher amounts of DKN and BA in leachate compared with any of the grass treatments (Table 2 and Fig. 3). However, grass treatments did not significantly enhance the degradation of DKN into BA (Table 2). This result implied that (i) DKN degradation is abiotic and rhizosphere soil microorganisms do not play an important role in its degradation, (ii) grass treatments do not create a rhizosphere environment that enhances DKN degradation, and/or (iii) plant uptake of DKN and BA significantly reduced the levels of these metabolites that were transported through the lysimeters.

In contrast to IXF, grass treatments receiving ATR did not significantly reduce the total ATR loss to the leachate (Table 1), but three of the grass treatments significantly increased the proportion of ATR metabolites present in the leachate (Table 1 and Fig. 4). Orchardgrass had the lowest amount of parent ATR in the leachate, with ATR transport reduced by 55% compared with the control. The HA levels were similar for all treatments (Table 1). Orchardgrass also had the

Table 3. Soil microbial biomass carbon of various ground treatments with atrazine or isoxaflutole (IXF) application.

Ground treatment	Biomass carbon		
	Atrazine	IXF	Mean ($p = 0.001$)
	g C/g soil		
Bare ground	65.7	69.0	67.3b†
Orchardgrass	187.5	126.7	157.1a
Tall fescue	189.7	135.0	162.3a
Timothy	140.7	140.3	140.5a
Smooth brome grass	171.3	145.3	158.3a
Switchgrass	169.3	151.3	160.3a
Mean ($p = 0.041$)‡	154.0a	127.9b	

† Means followed by the same letter within treatment factors do not differ significantly from each other at 5% level of probability using Fisher's LSD test.

‡ Interaction between effects of herbicide and ground cover types was not significant ($p = 0.56$).

highest proportion of metabolites to total ATR loss in leachate of any treatment, showing a 71% increase compared with the control treatment. Timothy and smooth brome grass treatments also showed significant increases in the proportion of ATR metabolites in leachate compared with the control (Table 1). However, the high quantity of ATR present in the leachate for the smooth brome grass treatments makes this species a poor choice for reducing transport of ATR to ground water. In general, the proportion of ATR metabolites in leachate of the grass treatments tended to become more significant over time (Fig. 4). From a remediation standpoint, the higher proportion of metabolites to total ATR in leachate provides a useful measure of the capacity of forage species to mitigate herbicide contamination of ground water. However, the forage species did not reduce total ATR transport to leachate compared with the control, suggesting that the primary mechanism for bioremediation was via enhanced rhizosphere microbial activity. One method of testing this hypothesis was to measure the microbial biomass carbon in the lysimeter soils.

Microbial Biomass Carbon versus Degradation

All grass treatments showed significantly higher microbial biomass carbon than that of the bare ground, but there was no significant effect of any specific forage ($p = 0.041$; Table 3). Regardless of grass treatments, the ATR-treated lysimeters had significantly greater microbial biomass carbon than IXF-treated lysimeters (Table 3). When all lysimeter data for ATR were pooled, the proportion of metabolites in leachate was significantly correlated with increased soil microbial biomass carbon ($r = 0.64$, $p = 0.0082$; Fig. 6A). This result suggested that enhanced biological degradation of ATR by grass treatments was probably due to the increased population and activity of ATR degraders in the rhizosphere. However, enumeration of ATR-degrading bacteria would be required to confirm this conclusion.

In contrast, the increased microbial biomass carbon in grass treatments had no significant effect on degrading the biologically active metabolite DKN to BA ($r = -0.29$, $p = 0.268$; Fig. 6B). The poor correlation between microbial biomass carbon and DKN degradation sup-

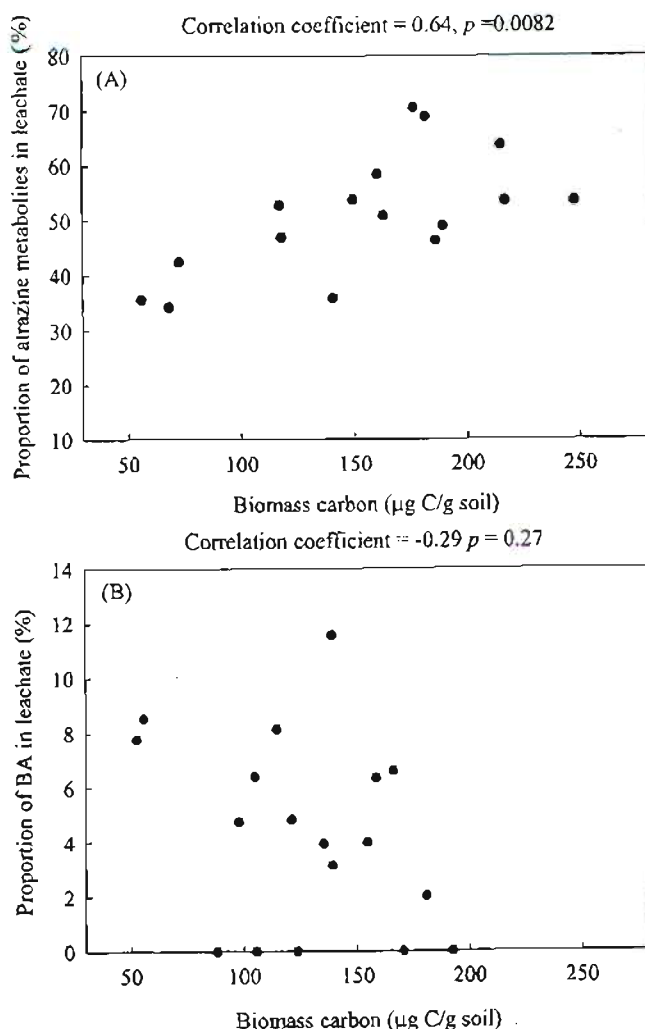


Fig. 6. (A) Microbial biomass carbon versus atrazine degradation and (B) diketonitrile degradation to benzoic acid.

ports the previous assertion that DKN hydrolysis is an abiotic process. Mougin et al. (2000) reported slow hydrolysis of DKN to BA by two extracellular fungal oxidases (lignin peroxidase and manganese-dependent peroxidase), suggesting a limited biological role in DKN hydrolysis. Rigorous testing of this hypothesis will require further enzyme assays and microbial studies.

CONCLUSIONS

The results of this study demonstrated that IXF is highly unstable. Within 32 h of application, leachate samples contained almost 100% DKN, the biologically active form of IXF. Diketonitrile showed a higher mobility during early rainfall events and less degradation than ATR. Grass treatments did not promote the hydrolysis of DKN, but they significantly reduce the total quantity of IXF metabolites present in the leachate through enhanced evapotranspiration. Although the grass treatments were not able to reduce the total ATR (ATR + DEA + DIA + HA) transported in the leachate, they significantly enhanced the degradation of ATR, particularly through N-dealkylation reactions. For ATR treatments, the proportion of metabolites in the leachate

strongly correlated with the elevated soil microbial biomass carbon in forage treatments. In contrast, DKN degradation was poorly correlated with soil microbial biomass carbon. Among the grass treatments, orchardgrass had the least parent ATR transport and highest proportion of ATR metabolites in the leachate. Timothy and smooth bromegrass treatments also significantly increased the proportion of ATR metabolites present in leachate.

The mechanism by which grasses mitigate transport of herbicides to ground water differs based on the herbicide chemistry. For IXF, mitigation was achieved through enhanced evapotranspiration reducing the volume of percolating water. For atrazine, mitigation occurred by enhanced transformation of ATR into less-toxic metabolites. From a management standpoint, field implementation of grass buffers with any of the species tested would reduce transport of DKN to ground water, but for ATR, orchardgrass or timothy would be the best choices for ground water mitigation.

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